Amendments to the Claims

This listing of claims replaces all prior versions, and listings, of claims in the above-identified application:

1. (currently amended) A method for attenuating the expression of a target gene in a ~~vertebrate~~ zebrafish cell *in vivo* comprising supplying the cell with a double stranded RNA in an amount sufficient to specifically attenuate expression of the target gene, wherein one of the strands of the double stranded RNA is capable of hybridizing to the target gene *in vitro* in 400 mM NaCl, 40 mM PIPES pH 6.4, and 1 mM EDTA, at 50°C, and provided that, when the double stranded RNA is supplied to the cell by delivery to the cell of double stranded RNA, the double stranded RNA is formed from single-stranded RNA that is purified in the absence of phenol or chloroform.

2. (original) The method of claim 1 wherein the target gene is an endogenous gene.

3. (original) The method of claim 1 wherein the target gene is a foreign gene.

4. (previously presented) The method of claim 1 wherein the target gene is a chromosomal gene.

5. (previously presented) The method of claim 1 wherein the target gene is an extrachromosomal gene.

6. (currently amended) The method of claim 1 wherein the target gene is from a pathogen capable of infecting the zebrafish cell.

7. (original) The method of claim 6 wherein the pathogen is selected from the group consisting of a virus, bacterium, fungus or protozoan.

8-14 (canceled)

15. (previously presented) The method of claim 1 wherein the double stranded RNA comprises a nucleotide sequence that is complementary to the nucleotide sequence of at least a portion of the target gene.

16. (previously presented) The method of claim 1 wherein the double stranded RNA comprises a nucleotide sequence that is complementary to a region of at least about 25 bases of the target gene.

17. (previously presented) The method of claim 1 wherein the double stranded RNA is supplied in an amount sufficient to completely inhibit expression of the target gene.

18. (previously presented) The method of claim 1 in which the double stranded RNA comprises a single strand comprising self-complementary portions.

19. (original) The method of claim 1 in which the double stranded RNA comprises two separate complementary strands.

20. (currently amended) The method of claim 1 wherein the zebrafish cell is an embryonic cell.

21. (currently amended) A method for attenuating the expression of a target gene in an embryonic fish cell *in vivo* comprising supplying the cell with a double stranded RNA in

an amount sufficient to specifically attenuate expression of the target gene, wherein one of the strands of the double stranded RNA is capable of hybridizing to the target gene *in vitro* in 400 mM NaCl, 40 mM PIPES pH 6.4, and 1 mM EDTA, at 50°C, and provided that, when the double stranded RNA is supplied to the cell by delivery to the cell of double stranded RNA, the double stranded RNA is formed from single-stranded RNA that is purified in the absence of phenol or chloroform. ~~The method of claim 20 wherein the embryonic cell is a fish embryonic cell.~~

22. (currently amended) The method of claim 20 wherein the embryonic zebrafish cell is supplied with the double stranded RNA using microinjection.

23. (currently amended) The method of claim 1 wherein the zebrafish cell is present in a tissue, an organ, or an organism.

24. (currently amended) The method of claim 23 wherein the zebrafish cell is present in an organism, and the cell is supplied with the double stranded RNA by introducing double stranded RNA into a body cavity or interstitial space of the organism.

25, 26 (canceled)

27. (currently amended) The method of claim 1 wherein supplying the double stranded RNA to the zebrafish cell comprises delivering double-stranded RNA to the zebrafish cell, and wherein the double stranded RNA is treated with RNAse prior to delivery to the zebrafish cell.

28. (currently amended) The method of claim 1 wherein supplying the double stranded RNA to the zebrafish cell comprises delivering double stranded RNA to the zebrafish cell, the method

further comprising, prior to delivering the double stranded RNA to the zebrafish cell, annealing two complementary single stranded RNAs to yield the double stranded RNA.

29. (previously presented) The method of claim 28 wherein the single stranded RNAs are annealed in the presence of potassium chloride.

30. (original) The method of claim 1 wherein the function of the target gene is unknown.

31. (currently amended) The method of claim 1 further comprising introducing into the zebrafish cell a second double stranded RNA in an amount sufficient to attenuate expression of a second target gene, wherein one of the strands of the second double stranded RNA is capable of hybridizing to the second target gene *in vitro* in 400 mM NaCl, 40 mM PIPES pH 6.4, and 1 mM EDTA, at 50°C .

32. (currently amended) The method of claim 1 comprising introducing into the zebrafish cell multiple double stranded RNAs in an amount sufficient to attenuate expression of multiple target genes, wherein one strand of each double stranded RNA is capable of hybridizing to the corresponding target gene *in vitro* in 400 mM NaCl, 40 mM PIPES pH 6.4, and 1 mM EDTA, at 50°C.

33-38. (canceled)

39. (previously presented) The method of claim 23 further comprising identifying a phenotypic change in the organism associated with attenuated expression of the target gene.

40-47. (canceled)

48. (currently amended) A method for attenuating the expression of a target gene in a ~~vertebrate~~ zebrafish cell comprising:

annealing two complementary single stranded RNAs in the presence of potassium chloride to yield double stranded RNA;

contacting the double stranded RNA with RNAse to purify the double stranded RNA by removing single stranded RNA; and

introducing the purified double stranded RNA into the cell in an amount sufficient to specifically attenuate expression of the target gene;

wherein one of the strands of the double stranded RNA is capable of hybridizing to the target gene *in vitro* in 400 mM NaCl, 40 mM PIPES pH 6.4, and 1 mM EDTA, at 50°C, and wherein the double stranded RNA is formed from single-stranded RNA that is purified in the absence of phenol or chloroform.

49-61. (canceled)

62. (previously presented) The method of claim 1 wherein one of the strands of the double stranded RNA is capable of hybridizing to the target gene *in vitro* in 400 mM NaCl, 40 mM PIPES pH 6.4, and 1 mM EDTA, at 70°C.

63. (currently amended) A method for attenuating the expression of a target gene in a ~~vertebrate~~ zebrafish cell *in vivo* comprising delivering a double stranded RNA to the zebrafish cell in an amount sufficient to specifically attenuate expression of the target gene, wherein the double stranded RNA comprises a nucleotide sequence that is complementary to a region of at least 25 nucleotides of the target gene, and wherein the double stranded RNA is formed from single-stranded RNA that is purified in the absence of phenol or chloroform.

64-71 (canceled)

72. (currently amended) The method of claim 63 ~~or 65~~ wherein the target gene is associated with a disease.

73. (currently amended) The method of claim 63 ~~or 65~~ wherein the target gene is associated with a disease from a pathogen.

74. (currently amended) The method of claim 1~~, 64, 65 or 66~~ wherein the double stranded RNA is supplied to the zebrafish cell by delivering to the cell a DNA encoding the double stranded RNA.

75. (previously presented) A method for attenuating the expression of a target gene in a vertebrate cell *ex vivo* comprising:

explanting a cell from a vertebrate organism;

supplying the cell with a double stranded RNA in an amount sufficient to specifically attenuate expression of the target gene, wherein one of the strands of the double stranded RNA is capable of hybridizing to the target gene in vitro in 400 mM NaCl, 40 mM PIPES pH 6.4, and 1 mM EDTA, at 50°C, and provided that, when the double stranded RNA is supplied to the cell by delivery to the cell of double stranded RNA, the double stranded RNA is formed from single-stranded RNA that is purified in the absence of phenol or chloroform; and

implanting the cell into a vertebrate organism.

76. (previously presented) The method of claim 75, wherein the cell is implanted back into the vertebrate organism from which it was explanted.

77. (canceled)

78. (previously presented) The method of claim 1, wherein the double stranded RNA has a length of less than about 200 bases.

79. (previously presented) The method of claim 18 wherein the double stranded RNA comprises a nucleotide sequence that is complementary to a region of at least about 25 bases of the target gene.

80. (previously presented) The method of claim 19 wherein the double stranded RNA comprises a nucleotide sequence that is complementary to a region of at least about 25 bases of the target gene